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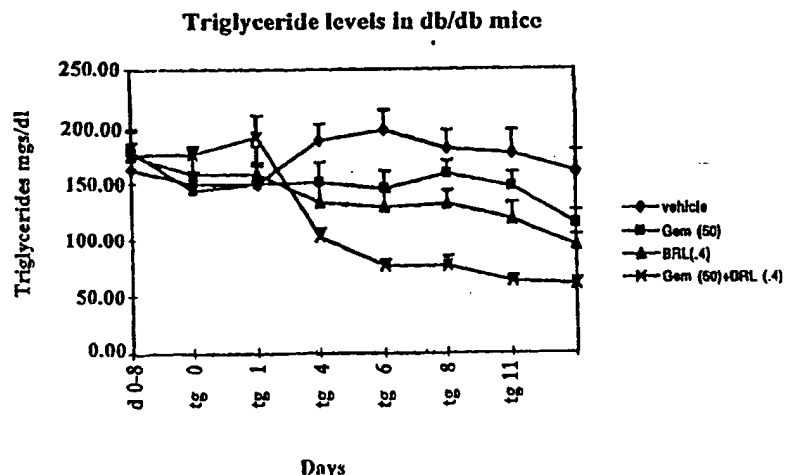
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International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>A61K 31/45, 31/19</b>		<b>A2</b>	(11) International Publication Number: <b>WO 98/05331</b>
			(43) International Publication Date: 12 February 1998 (12.02.98)
(21) International Application Number: <b>PCT/US97/13605</b>		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 1 August 1997 (01.08.97)		<b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>	
(30) Priority Data: 60/022,949 2 August 1996 (02.08.96) US			
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(54) Title: **PREVENTION OR TREATMENT OF TYPE 2 DIABETES OR CARDIOVASCULAR DISEASE WITH PPAR MODULATORS**



(57) Abstract

This invention relates to methods and compositions for the prevention and treatment of Type 2 diabetes and cardiovascular disease with diabetic or pre-diabetic conditions or symptoms associated therewith using both a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist or a compound which activates both PPAR $\gamma$  and PPAR $\alpha$ . A preferred PPAR $\gamma$  agonist is a thiazolidinedione compound, including BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075, darglitazone, and congeners, analogs, derivatives and pharmaceutically acceptable salts thereof. A preferred PPAR $\alpha$  agonist is a fibrate compound including gemfibrozil, fenfibrate, bezofibrate, clofibrate, ciprofibrate, and analogs, derivatives and pharmaceutically acceptable salts thereof.

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PREVENTION OR TREATMENT OF TYPE 2 DIABETES OR CARDIOVASCULAR  
DISEASE WITH PPAR MODULATORS

Field of the Invention

5           This invention relates to methods and pharmaceutical  
compounds for treating diabetes or cardiovascular diseases.

Background of the Invention

10           Non-insulin-dependent diabetes mellitus (NIDDM or  
Type 2 diabetes) is characterized by abnormalities in insulin  
secretion and action. Over 90% of the approximately six  
million diagnosed diabetics in the United States have Type 2  
diabetes.

15           The hallmark of Type 2 diabetes is insulin  
resistance, which is manifested as a decrease of insulin-  
stimulated glucose uptake in skeletal muscle and adipose  
tissue. Insulin resistance is also seen in decreased ability  
of insulin to suppress hepatic glucose output, and abnormal  
insulin secretion by pancreas. These impairments of insulin  
20   action are central to the development of elevated fasting  
blood glucose level and glucose intolerance.

          Impaired insulin action also leads to elevated  
plasma insulin level (hyperinsulinemia), and is frequently  
associated with hypertension, elevated bodyweight, elevated  
25   levels of triglycerides, uric acid, fibrinogen, small dense  
LDL (Low Density Lipoprotein) particles, and plasminogen  
activator inhibitor 1 (PAI-1), and decreased levels of HDL  
(High Density Lipoprotein). This constellation of metabolic  
abnormalities has been termed "Syndrome X." Each of these

individual changes is associated with an increase in the risk of developing coronary artery disease (CAD). In combination, they represent a strongly atherogenic profile that explains the greatly increased incidence of CAD documented in patients with Type 2 diabetes.

Diet and exercise are first-line therapies for controlling blood glucose level in Type 2 diabetes patients. Type 2 diabetes patients also take oral hypoglycemic agents such as sulfonylurea insulin secretagogues, often in combination with insulin injection.

A major drawback of current drug therapies is the occurrence of potentially life-threatening hypoglycemia due to hyperinsulinemia. In addition, existing drugs often fail to normalize Type 2 diabetes associated plasma lipid abnormalities that lead to cardiac morbidity and mortality. Therefore, a need exists for antidiabetic therapies that do not produce hypoglycemia, and are effective in ameliorating diabetic dyslipidemia.

Thiazolidinediones (TZDs) have been documented to exert antihyperglycemic activity by increasing insulin action rather than promoting insulin secretion (Hofmann, C. A. and J. R. Colca (1992) Diabetes Care 15: 1075-1078). Thiazolidinediones ameliorate insulin resistance and normalize plasma insulin and glucose levels without causing hypoglycemia. The thiazolidinedione insulin sensitizers, e.g. ciglitazone, englitazone, pioglitazone, BRL 49653, and troglitazone, enhance insulin-mediated suppression of hepatic glucose output and insulin-stimulated glucose uptake by

skeletal muscle and adipose tissue. Thiazolidinediones also lower the level of triglycerides and elevate the level of HDL.

Fibrates have been documented to lower plasma triglycerides and cholesterol levels and to be beneficial in the prevention of ischemic heart disease in individuals with elevated levels of LDL cholesterol. They can also modestly decrease elevated fibrinogen and PAI-1 levels. Fibrate compounds, e.g., gemfibrozil, fenofibrate, bezafibrate, and ciprofibrate, elevate the level of plasma HDL cholesterol.

Abbreviations used in this application are described below: Apo = Apolipoprotein; ACO = Acyl CoA oxidase; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; VLDL = Very Low Density Lipoprotein; PPAR = Peroxisome Proliferator Activated Receptor; and PAI-1 = Plasminogen Activator Inhibitor - 1.

#### Summary of the Invention

The compositions and methods of this invention are useful for treating, curing, reducing, or preventing one or more clinical symptoms of or associated with Type 2 diabetes and cardiovascular disease with diabetic or pre-diabetic conditions, including, but not limited to, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, high fibrinogen, high PAI-1, low HDLc, high LDLc, hypertension and obesity.

Type 2 diabetic patients are often hyperlipidemic and face increased risk for coronary heart disease. The most frequent lipid abnormality encountered in Type 2 diabetes is hypertriglyceridemia associated with reduced levels of HDL and the presence of small dense LDL. Therefore, pharmacological

treatment of these patients should aim not only at normalizing glucose metabolism, but also at correcting lipid abnormalities.

Pre-diabetic subjects are individuals with fasting  
5 plasma glucose levels between about 110 and 125 mg/dL. As of  
June 1997, the American Diabetes Association recognizes these  
individuals as having "impaired fasting glucose." Insulin  
resistance is most likely present in these individuals. The  
pre-diabetic subjects are at high risk for subsequent  
10 development of Type 2 diabetes (where fasting plasma glucose  
levels are at or above 126 mg/dL). These individuals are  
predisposed to the development of cardiovascular disease.  
Normalizing glucose and lipid metabolism in these subjects  
helps to prevent the development of Type 2 diabetes and  
15 cardiovascular disease.

Within the scope of this invention, Applicant has  
discovered synergistic effects of a PPAR $\gamma$  agonist and a PPAR $\alpha$   
agonist in preventing or ameliorating the symptoms of or  
associated with Type 2 diabetes and cardiovascular disease  
20 with diabetic or pre-diabetic conditions. For example,  
Applicant has shown that co-administration of suboptimal doses  
of a PPAR $\gamma$  agonist (e.g., a thiazolidinedione) and a PPAR $\alpha$   
agonist (e.g., a fibrate) lowered triglyceride concentrations  
more effectively than either agent alone in animal models of  
25 Type 2 diabetes. Such combination therapy thus allows one to  
use lower doses of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist to  
avoid or reduce their respective toxicity to patients without  
compromising their antidiabetic and cardio-protective effects.  
In addition, co-administration of a PPAR $\gamma$  agonist and a PPAR $\alpha$

agonist can reduce triglycerides to levels either agent alone cannot achieve. Furthermore, the combination therapy can control multiple diabetic and cardiovascular risk factors at the same time by (1) normalizing elevated levels of plasma triglyceride, glucose and insulin, and (2) correcting lipid and hemostatic abnormalities by elevating the level of HDLc, lowering the levels of small dense LDL and fibrinogen, and reducing body weight and blood pressure. Such combination treatment is more effective than treatments with one type of agent alone.

Therefore, the present invention relates to methods and compositions for preventing or treating Type 2 diabetes or cardiovascular disease with diabetic or pre-diabetic conditions in a patient host by administering to the host a composition containing a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist. The host may be a human patient or an animal, e.g., mammals such as mice, rats, horses, pigs, and dogs, including animal models of Type 2 diabetes and cardiovascular disease with diabetic or pre-diabetic conditions. The compositions of this invention are adapted to cure, treat, improve or prevent one or more symptoms of Type 2 diabetes and cardiovascular disease with diabetic or pre-diabetic conditions in the host. A preferred composition is highly potent and selective with low toxicity. The compositions of this invention may be in a single dosage unit optionally containing a pharmaceutically acceptable carrier or excipient. In this regard, those skilled in the art will recognize Type 2 diabetes as an example of a metabolic disease that can be treated with the methods and

compositions of the present invention. Other metabolic disorders relating to the levels of glucose, triglyceride, and other lipids, or relating to hypertension, obesity and disorders of blood coagulation can also be treated with the methods and compositions of the present invention.

In addition, the methods and compositions of this invention can be used to increase the level of HDL cholesterol (preferable by 50%, more preferable by two fold), increase insulin sensitivity (preferable by 50%, more preferable by two fold), increase glucose uptake in the adipose or muscle tissue (preferable by 50%, more preferable by two fold), lower the level of plasma triglyceride (preferable by 10%, more preferable by 30%), lower the level of plasma glucose (preferable by 10%, more preferable by 30%), lower the level of plasma insulin (preferable by 10%, more preferable by 30%), lower the body weight (preferable by 10%, more preferable by 30%), lower the blood pressure (preferable by 10%, more preferable by 30%), or lower the level of plasma fibrinogen (preferable by 10%, more preferable by 30%) by administering to a host an amount of a PPAR agonist and a PPAR agonist to achieve the desired effect.

By "pharmaceutically effective amount" is meant an amount of a pharmaceutical compound or composition having a therapeutically relevant effect on Type 2 diabetes, cardiovascular disease with diabetic or pre-diabetic conditions, or other metabolic disorders. A therapeutically relevant effect relieves to some extent one or more symptoms of these diseases in a patient or returns to normal either partially or completely one or more physiological or



biochemical parameters associated with or causative of these diseases. Examples of therapeutically relevant effects include: increasing the sensitivity of cellular response to circulating insulin, curing, treating, reducing, or preventing one or more clinical symptoms of Type 2 diabetes and cardiovascular disease with diabetic or pre-diabetic conditions, including, but not limited to, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, high fibrinogen, high PAI-1, low HDLc, high LDLc, hypertension and obesity. In a preferred embodiment, a pharmaceutically effective amount of a compound or composition means an amount that decreases the level of plasma glucose. In another preferred embodiment, a pharmaceutically effective amount of a compound or composition means an amount that decreases the level of plasma triglycerides. In yet another preferred embodiment, a pharmaceutically effective amount of a compound or composition means an amount that increases the plasma HDLc level, decreases the plasma LDLc level, reduces the presence of small dense LDL, or does any combination of the above.

By "PPAR $\gamma$  agonist" is meant a compound or composition which when combined with PPAR $\gamma$  directly or indirectly (preferably binding directly to PPAR $\gamma$ ) stimulates or increases an *in vivo* or *in vitro* reaction typical for the receptor, e.g., transcriptional regulation activity, as measured by an assay known to one skilled in the art, including, but not limited to, the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355, WO91/06677, WO92/05447, WO93/11235, WO95/18380,

PCT/US93/04399, PCT/US94/03795, CA 2,034,220, and Lehmann, et al., J. Biol. Chem. 270:12953-12956 (1995), which are incorporated by reference herein. A preferred PPAR $\gamma$  agonist is a thiazolidinedione compound, including, but not limited to, BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075, darglitazone, and congeners, analogs, derivatives and pharmaceutically acceptable salts thereof. PPAR $\gamma$  compounds disclosed in Tontonez et al., Genes & Develop. 8:1224-1234 (1994), Tontonez et al., Cell 79:1147-1156 (1994), Lehmann et al., J. Biol. Chem. 270(22):1-4, 1995, Amri et al., J. Lipid Res. 32:1449-1456 (1991), Kliewer et al., Proc. Natl. Acad. Sci. USA 94:4318-4323 (1997), Amri et al., J. Lipid Res. 32:1457-1463, (1991) and Grimaldi et al., Proc. Natl. Acad. Sci. USA 89:10930-10934 (1992) are incorporated by reference herein.

By "PPAR $\alpha$  agonist" is meant a compound or composition which when combined with PPAR $\alpha$  directly or indirectly (preferably binding directly to PPAR $\alpha$ ) stimulates or increases an *in vivo* or *in vitro* reaction typical for the receptor, e.g. transcriptional regulation activity, as measured by an assay known to one skilled in the art, including, but not limited to, the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355, WO91/06677, WO92/05447, WO93/11235, WO95/18380, PCT/US93/04399, PCT/US94/03795, CA 2,034,220, and Lehmann, et al., J. Biol. Chem. 270:12953-12956 (1995), which are incorporated by reference herein. A preferred PPAR $\alpha$  agonist is a fibrate compound including, but not limited to,

gemfibrozil, fenofibrate, bezofibrate, clofibrate, ciprofibrate, and analogs, derivatives and pharmaceutically acceptable salts thereof. PPAR $\alpha$  compounds disclosed in Tontonez et al., Genes & Develop. 8:1224-1234 (1994), Tontonez et al., Cell 79:1147-1156 (1994), Lehmann et al., J. Biol. Chem. 270(22):1-4, 1995, Amri et al., J. Lipid Res. 32:1449-1456 (1991), Kliewer et al., Proc. Natl. Acad. Sci. USA 94:4318-4323 (1997), Amri et al., J. Lipid Res. 32:1457-1463, (1991) and Grimaldi et al., Proc. Natl. Acad. Sci. USA 89:10930-10934 (1992) are incorporated by reference herein.

In a preferred embodiment, a single chemical entity that effectively activates both PPAR $\gamma$  and PPAR $\alpha$  is used, including, but not limited to, GW2331 (2-(4-[2,4-Difluorophenyl]-1-heptylureido)ethylphenoxy)-2-methylbutyric acid, see Kliewer et al., Proc. Natl. Acad. Sci. USA 94:4318-4323 (1997)) and analogs, derivatives and pharmaceutically acceptable salts thereof. GW2331 was synthesized by Ligand Pharmaceuticals and tested as described below for the combination of gemfibrozil and BRL 49653 and was found to significantly lower glucose and triglyceride levels in diabetic obese mice after seven days of treatment. Other candidate compounds can be assayed for the dual agonist activity using the "co-transfection" and "cis-trans" assays and ligand binding assays known to one skilled in the art or disclosed in this application. A compound so screened out is then provided in a pharmaceutically effective amount (optionally with a pharmaceutically acceptable carrier or excipient) for treating or preventing one or more clinical symptoms of or associated with Type 2 diabetes and

cardiovascular disease with diabetic or pre-diabetic conditions, including, but not limited to, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, high fibrinogen, high PAI-1, low HDLc, high LDLc, hypertension and obesity.

5 In another preferred embodiment, an RXR ligand is used in place of or in addition to the PPAR $\gamma$  agonist and/or PPAR $\alpha$  agonist in the composition or method of this invention.

By "RXR ligand" is meant a compound or composition which when combined with RXR (preferably binding directly to  
10 RXR) modulates, stimulates or increases *in vivo* or *in vitro* the transcriptional regulation activity of the RXR/PPAR $\gamma$  heterodimer and/or the RXR/PPAR $\alpha$  heterodimer, as measured by an assay known to one skilled in the art, including, but not  
15 limited to, the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355, WO91/06677, WO92/05447, WO93/11235, WO95/18380, PCT/US93/04399, PCT/US94/03795 and CA 2,034,220, which are incorporated by reference herein. It includes RXR agonists which when  
20 combined with RXR homodimers or heterodimers increase the transcriptional regulation activity of both the RXR homodimers and heterodimers. It also includes RXR antagonists which increase the transcriptional regulation activity of RXR/PPAR $\gamma$  heterodimers and/or RXR/PPAR $\alpha$  heterodimers and decrease the  
25 transcriptional regulation activity of RXR homodimers, including, but not limited to, compounds described or disclosed in PCT/US96/14876, LG100754 (*i.e.* (2E,4E,6Z)-3-methyl-7-[3-*n*-propoxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-octa-2,4,6-trienoic acid),

LG100823 (i.e. (2E,4E,6Z)-7-(3-benzyloxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-3-methylocta-2,4,6-trienoic acid), LG100541 (i.e. 4-(3,4,5,6,7,8-hexahydro-5,5,8,8-tetramethylantracen-1-ylmethyl)-benzoic acid), and  
5 cogeners, analogs, derivatives and pharmaceutically acceptable salts thereof. It includes, but is not limited to, compounds that preferentially activate RXR over RAR (i.e., RXR specific agonists), and compounds that activate both RXR and RAR (i.e., pan agonists). RXR specific ligands include, but are not  
10 limited to, LG 100268 (i.e. 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-cyclopropyl]-pyridine-5-carboxylic acid) and LGD 1069 (i.e. 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-2-carbonyl]-benzoic acid), and  
congeners, analogs, derivatives and pharmaceutically  
15 acceptable salts thereof. The structures and syntheses of LG 100268 and LGD 1069 are disclosed in Boehm, et al. J. Med. Chem. 38(16):3146-3155, 1994, incorporated by reference herein. Pan agonists include, but are not limited to, ALRT  
1057 (i.e. 9-cis retinoic acid), and analogs, derivatives and  
20 pharmaceutically acceptable salts thereof. The entire content of WO 97/10819 by Heyman et al. is incorporated by reference herein, including the references, drawings and sequences described or disclosed therein. Preferably, RXR specific  
agonists are selected for reduced side effects in comparison  
25 to pan agonists. It also includes compounds that activate RXR in a certain cellular context but not others (i.e., RXR partial agonists). Compounds disclosed or described in the following articles, patents and patent applications which have RXR agonist activity are incorporated by reference herein:

U.S. patents 5,399,586 and 5,466,861, WO96/05165,  
PCT/US95/16842, PCT/US95/16695, PCT/US93/10094, WO94/15901,  
PCT/US92/11214, WO93/11755, PCT/US93/10166, PCT/US93/10204,  
WO94/15902, PCT/US93/03944, WO93/21146, PCT/US96/14876, Boehm,  
5 et al. J. Med. Chem. 38(16):3146-3155, 1994, Boehm, et al. J.  
Med. Chem. 37(18):2930-2941, 1994, Antras et al., J. Biol.  
Chem. 266:1157-1161 (1991), Salazar-Olivo et al., Biochem.  
Biophys. Res. Commun. 204:157-263 (1994) and Safanova, Mol.  
Cell. Endocrin. 104:201-211 (1994).

10           The combination of a PPAR $\gamma$  agonist and a PPAR $\alpha$   
agonist may also be used with other PPAR subtype modulators  
(e.g., PPAR $\beta$  or NUC1 modulators) which give a similar  
beneficial treatment profile. For example, NUC1 is known to  
repress the activity of PPAR $\alpha$  and PPAR $\gamma$ . Therefore, an agent  
15 which reduces or relieves the repression of PPAR $\alpha$  or PPAR $\gamma$  by  
PPAR $\beta$  or NUC1 is useful in enhancing the triglyceride lowering  
activity of a PPAR $\alpha$  or PPAR $\gamma$  agonist. Such an agent may be  
used either alone or in combination with a PPAR $\alpha$  or PPAR $\gamma$   
agonist to treat Type 2 diabetes and/or cardiovascular disease  
20 with diabetic or pre-diabetic conditions.

          Therefore, in a preferred embodiment, the  
pharmaceutical composition contains a pharmaceutically  
effective amount of a PPAR $\beta$  or NUC1 modulator which reduces or  
relieves the repression of PPAR $\alpha$  or PPAR $\gamma$  by PPAR $\beta$  or NUC1.  
25 The disclosure of published PCT application WO 96/01430 is  
incorporated by reference herein, including the sequences and  
drawings therein.

          An alternative to administering to the host a  
composition containing a pharmaceutically effective amount of

a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist is to administer to the host two compositions, one containing a pharmaceutically effective amount of a PPAR $\gamma$  agonist and the other containing a pharmaceutically effective amount of a PPAR $\alpha$  agonist.

5           In a further preferred embodiment, the pharmaceutical composition also contains a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizier, insulin mimetic, metformin, acarbose, or sulfonyl ureas. Alternatively, a composition  
10           containing a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizier, or insulin mimetic is administered to the host separately.

          A composition containing a pharmaceutically effective amount of an active ingredient may be administered  
15           systemically to a host. In a preferred embodiment, it is administered orally. Other routes of administration are described in a section entitled Pharmaceutical Formulations and Modes of Administration below.

          In another aspect, this invention features a  
20           pharmaceutical composition for treating Type 2 diabetes or cardiovascular disease with diabetic or pre-diabetic conditions in a patient host wherein the composition contains (1) a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist; and (2) a pharmaceutically acceptable carrier  
25           adapted for the host. In a preferred embodiment, the pharmaceutical composition also includes a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizier, insulin mimetic, metformin, acarbose, or sulfonyl ureas.

In a preferred embodiment, the composition is held within a container which includes a label stating to the effect that the composition is approved by the FDA in the United States (or an equivalent regulatory agency in a foreign country) for treating Type 2 diabetes or cardiovascular disease or for treating hyperglycemia, hyperinsulinemia, hypertriglyceridemia, high LDLc, low HDLc, high PAI-1, hypertension, obesity, or hyperfibrinogenemia. Such a container provides a therapeutically effective amount of the active ingredient to be administered to a host.

Other features and advantages of the invention will be apparent from the following detailed description of the invention, and from the claims.

#### Brief Description of the Drawings

Figure 1 is a graph showing the levels of plasma triglyceride in db/db diabetic mice treated for 14 days with a PPAR $\alpha$  agonist (gemfibrozil), a PPAR $\gamma$  agonist (BRL 49653), or the agents in combination. Gemfibrozil was administered at 50 mg/kg/day, BRL 49653 was administered at 0.4 mg/kg/day.

Figure 2 (A-D) is a graph showing the distribution of triglyceride levels within the plasma lipoproteins of normal chow-fed rats treated for 14 days with a PPAR $\alpha$  agonist (fenofibric acid), a PPAR $\gamma$  agonist (thiazolidinedione compound BRL 49653), or the agents in combination. Fenofibric acid was administered at 40 mg/kg/day, BRL 49653 was administered at 5 mg/kg/day.



Detailed Description of the InventionLipid normalizing effects of fibrates occur through activation of PPAR $\alpha$ 

Fibrates are lipid altering agents that reduce triglyceride levels in hypertriglyceridemic patients and animals, including animal models with normal or elevated levels of triglycerides. Administration of fenofibrate, bezafibrate or gemfibrozil to rats lowers triglyceride levels.

Applicant has found that the lipid-lowering effects of the widely used hypolipidemic drugs, fibrates, are mediated through activation of PPAR and subsequent changes in the expression of genes involved in lipoprotein metabolism both in rodents as well as in humans (for review see Schoonjans, K., et al. (1996) J. Lipid Res. 37: 907-925, not admitted to be prior art). PPARs form heterodimers with RXR and the heterodimers bind to specific response elements, termed PPRES, in the regulatory regions of target genes and subsequently alter their transcription. The majority of the genes whose expression is under control of PPARs code for proteins involved in intra- and extracellular lipid metabolism, such as the enzymes of the peroxisomal and mitochondrial  $\beta$ -oxidation pathways, 3-hydroxy-3-methylglutaryl-coA synthase, adipocyte fatty acid binding protein aP2, acyl-coA synthetase and apolipoproteins A-I, A-II and C-III.

Fibrates are PPAR $\alpha$  agonists. PPAR $\alpha$  is a member of the nuclear receptor superfamily of transcription factors. PPAR $\alpha$  binds a regulatory element identified as C3P on the promoter of the apo CIII gene, which encodes apolipoprotein CIII. Apolipoprotein CIII modulates triglyceride levels in

plasma. Mice that genetically overexpress apo CIII have higher than normal triglyceride levels. Mice made defective in the expression of apo CIII through genetic knockout techniques have lower than normal triglyceride levels.

5           Applicant's findings indicate that fibrates, such as fenofibrate, have a predominant effect on apolipoprotein gene expression in liver, but not in other tissues, such as adipose tissue. Applicant's data show that fibrates decrease hepatic production of apolipoprotein (apo) CIII by lowering the level  
10 of apo CIII mRNA. The effects of fibrates on plasma high density lipoprotein (HDL) concentrations are, at least partially, due to a PPAR-mediated transcriptional regulation of the major HDL apolipoproteins, apo A-I, apo A-II and apo A-IV, in liver. Along the same line, the hypotriglyceridemic  
15 action of fibrates can be attributed to alterations in the expression of specific genes in liver, leading to an increased lipolysis of triglyceride-rich lipoproteins and clearance of remnant particles from plasma. This latter effect is at least partially mediated by apo C-III. Indeed, fibrates have been  
20 shown to down regulate the expression of apo C-III in liver, but not in other tissues such as intestine (apo C-III), heart (LPL) or adipose tissue (LPL).

25           Thiazolidinediones achieves antidiabetic effects through activation of PPAR $\gamma$

Thiazolidinediones are insulin sensitizers that significantly reduce glucose and triglyceride levels in Type 2 diabetes and cardiovascular disease patients and animal models of Type 2 diabetes (Kees, et al., J. Medicinal Chem.

38(4):617-628, 1995; Willson, et al., J. Medicinal Chem.  
39(3):665-668, 1996; Young, et al. Diabetes 44:1087-1092,  
1995). Thiazolidinediones improve glucose utilization without  
stimulating insulin release.

5           These compounds improve glucose metabolism by  
increasing peripheral insulin sensitivity as demonstrated in  
animal models of insulin resistance, such as the genetically  
obese *ob/ob* mice and Zucker *fa/fa* rats or genetically diabetic  
*db/db* or yellow KKA mice (Young, P. W., et al. (1995) Diabetes  
10 44: 1087-1092; Nolan, J. J., et al. (1994) New Engl. Med. 331:  
1188-1193). Treatment of these animals with different  
thiazolidinediones, such as ciglitazone, troglitazone (CS-  
045), pioglitazone and englitazone, results in markedly  
improved glucose tolerance. Similarly, administration of  
15 troglitazone to humans resulted in reduced insulin and glucose  
levels and improved insulin sensitivity, as measured by oral  
glucose tolerance test and euglycemic-hyperinsulinemic clamp  
studies. Administration of BRL 49653 to *ob/ob* mice improves  
glycemic control by increasing insulin responsiveness of  
20 target tissues. BRL 49653 potentiates insulin-stimulated  
glucose transport in adipocytes from insulin-resistant obese  
mice both by increasing insulin receptor number and by  
facilitating translocation of GLUT4 from an expanded  
intracellular pool to the cell surface.

25           In addition to their effects on plasma glucose  
metabolism, thiazolidinediones are hypolipidemic agents,  
decreasing plasma triglyceride concentrations both in diabetic  
and normal rodents, primates and humans (Kemnitz, J. W., et  
al. (1994) Diabetes 43: 204-211).

Thiazolidinediones are also selective PPAR $\gamma$  agonists (Lehmann, et al. J. Biol. Chem. 270(22):1-4, 1995).

Comparison of the EC<sub>50</sub> for activation of PPAR $\gamma$  with the minimum effective dose for glucose lowering activity revealed a  
5 significant correlation. The correlation between *in vitro* PPAR $\gamma$  activity and *in vivo* glucose lowering activity of thiazolidinediones implicates PPAR $\gamma$  as the molecular target for the antidiabetic effects of thiazolidinediones.

PPAR $\gamma$  is a member of the nuclear receptor  
10 superfamily of ligand-activated transcription factors. In contrast to PPAR $\alpha$ , which is predominantly expressed in tissues catabolizing high amounts of fatty acids, such as liver, heart and brown adipose tissue, PPAR $\gamma$  is predominantly expressed in white adipose tissue in rodents (Braissant, O., et al. (1995)  
15 Endocrinology 137: 354-366). Its expression is induced early during the course of differentiation of several preadipocyte cell lines. In fibroblasts, forced expression of PPAR $\gamma$  in the presence of an agonist such as a thiazolidinedione results in differentiation to an adipocyte phenotype.

20 Adipocytes are highly specialized cells that play a critical role in lipid metabolism and energy homeostasis. Their primary role is to store triglycerides in times of caloric excess and to mobilize this reserve during periods of nutritional deprivation. Adipogenesis plays a role in the  
25 development of Type 2 diabetes, which is characterized by not only unbalanced glucose homeostasis, but also elevated levels of circulating lipids. Increases in lipid levels have been shown to interfere with glucose disposal.

Adipocyte differentiation also involves the expression of other genes such as lipoprotein lipase (LPL) whose production promotes the accumulation of triglyceride in adipocytes. The gene for adipose tissue LPL contains a response element for PPAR $\gamma$  in its promoter. LPL activity increases in adipocytes when PPAR $\gamma$  is expressed and activated by compounds such as thiazolidinediones.

Applicant evaluated the influence of a potent thiazolidinedione, BRL49653, on serum lipoproteins and determined whether its lipid-lowering effects are mediated by changes in the expression of key-genes implicated in lipoprotein metabolism.

***Treatment with BRL49653 decreases serum triglyceride and VLDL concentrations without changing VLDL composition***

Since the hypotriglyceridemic action of TZDs is also observed in non-diabetic normal rats, Applicant studied the effects of the thiazolidinedione BRL49653 on lipoprotein metabolism in normolipidemic, normoglycemic rats, which allows evaluation of its effects on lipoprotein metabolism independent of its action on glucose homeostasis and which furthermore permits comparison of its mechanism of action to the well-described effects of fibrates in this animal model.

In order to study the effects of BRL49653 on serum lipids and lipoproteins, rats were treated with different doses of BRL49653 previously shown to affect lipid metabolism in the rat (Oakes, N. D., et al. (1994) Diabetes 43: 1203-1210). Administration of BRL49653 for 7 days did not change body or liver weights (Table 1). Epididymal fat pad weights,

however, increased in a dose-dependent fashion, an observation which is consistent with previously described inductive effects of thiazolidinediones on adipocyte differentiation and adipose tissue mass. Serum total, free and HDL cholesterol concentrations remained constant, whereas serum triglycerides decreased in a dose-dependent manner dropping to less than 50% of control at the highest dose tested (5 mg/kg/d) (Table 2).

In agreement with previous studies (Ikeda, H., et al. (1990) Drug Res. 40: 156-162; Oakes, N. D., et al. (1994) Diabetes 43: 1203-1210), serum glucose concentrations did not change after BRL49653 treatment (Table 2), confirming that thiazolidinediones do not exert a hypoglycemic action in the normoglycemic, non-diabetic rat. Separation of the different lipoprotein fractions by electrophoresis followed by lipostaining indicated that the changes in triglycerides after BRL49653 were reflected by a decrease in VLDL concentrations in serum. The decrease in VLDL was accompanied by an increase in IDL-LDL lipoproteins. By contrast, serum HDL lipid levels remained fairly constant, thereby confirming the unchanged HDL cholesterol concentrations after BRL49653 treatment (Table 2).

To determine whether BRL49653 treatment affected lipoprotein composition, rats were treated for 14 days with BRL49653 at doses from 5-20 mg/kg/d. Serum lipoproteins were separated by sequential ultracentrifugation and their composition analyzed next and expressed as a relative percentage (Table 3). Compared to control, VLDL particles isolated from rats treated with BRL49653 displayed a similar cholesterol, triglyceride, phospholipid and protein content

both at a dose of 5 mg/kg/d as well as at doses up to 20 mg/kg/d (Table 3).

In contrast, the relative triglyceride content of particles floating in the density interval between 1.006-1.063 (IDL + LDL) decreases by 25% after BRL49653 treatment at a dose of 5 mg/kg/d and up to 50% at 20 mg/kg/d (Table 3). The relative decrease in triglyceride content of the IDL+LDL particles was compensated by an enrichment in phospholipids, and, to a lesser extent, in total cholesterol (Table 3).

Although HDL triglycerides decreased after BRL49653 treatment, overall HDL composition was not significantly affected by BRL49653 treatment (Table 3).

Next, the apolipoprotein composition of the different lipoprotein fractions was analyzed by SDS-PAGE. Compared to control, treatment with BRL49653 did not induce major changes in either VLDL, IDL+LDL or HDL apolipoprotein distribution. In addition, comparison of the relative content of apo C-I, apo C-II and particularly apo C-III in VLDL by isoelectric focusing analysis did not show major changes between BRL49653 and controls. Altogether, these results indicate that BRL49653 lowers serum triglycerides by decreasing the number of VLDL particles without changing their composition in lipids or apolipoproteins.

#### ***BRL49653 treatment does not change the rate of triglyceride secretion***

Next, the mechanism behind the hypotriglyceridemic effect of BRL49653 was studied. First, it was analyzed whether BRL49653 treatment affects the production of

triglycerides *in vivo*. Injection with Triton WR-1339 blocks the clearance of triglyceride-rich lipoproteins by inhibiting their lipolytic degradation (Hirata, M. H., et al. (1987) Biochimica Biophysica Acta 917: 344-346) and thus allows an  
5 indirect measurement of triglyceride secretion rates by the measurement of serum triglycerides (Ishikawa, T. and N. Fidge (1979) J. Lipid Res. 20: 254-264). When rats treated for 7 days with BRL49653 (10 mg/kg/d) or vehicle were injected with Triton WR-1339 serum, triglyceride concentrations increased in  
10 a linear fashion (correlation coefficients of 0.977 and 0.967 for control and BRL49653 treated rats respectively). Calculation of the slopes of the curves indicated that the triglyceride secretion rates were not significantly different between control and BRL49653 treated rats (520±46 and 606±49  
15 mg/dl/h respectively), indicating that BRL49653 treatment does not act by changing triglyceride production.

***BRL49653 treatment increases adipose tissue LPL expression, without changing liver apolipoprotein gene expression***  
20

Next, Applicant studied the molecular mechanism of BRL49653 action and to compare it to the mechanism of fenofibrate, the most potent fibrate currently used for the treatment of hyperlipidemia. Since treatment of normal rats  
25 with BRL49653 appeared to affect triglyceride catabolism rather than triglyceride production, Applicant analyzed its effects on the expression of two major genes involved in plasma triglyceride catabolism, i.e. LPL and apo C-III. Whereas LPL, after activation by its co-factor apo C-II,



promotes the hydrolysis and clearance of triglyceride-rich lipoproteins in plasma, apo C-III antagonizes both these activities. Treatment of rats with BRL49653 did not change liver apo C-III mRNA levels at any of the doses tested. By contrast, epididymal adipose tissue LPL mRNA levels increased more than 2-fold after BRL49653 at doses of 5 and 10 mg/kg/d. The increase in LPL gene expression after BRL49653 was paralleled by a similar increase in LPL activity.

Since other PPAR activators, such as the fibrates fenofibrate, clofibrate and gemfibrozil as well as fish oil derived n-3 polyunsaturated fatty acids, have major effects on HDL apolipoprotein expression both in rodents (Staels, B., et al. (1990) Endocrinology 126: 2153-2163; Staels, B., et al. (1992) Arteriosclerosis and Thrombosis 12: 286-294) and in humans (Vu-Dac, N., et al. (1994) J. Biol. Chem. 269: 31012-31018; Vu-Dac, N., et al. (1995) J. Clin. Invest. 96: 741-750), the effects of BRL49653 on HDL apolipoprotein expression was investigated next.

Treatment of adult male rats with increasing doses of BRL49653 did not result in any significant changes in liver apo A-I, apo A-II and apo A-IV or serum apo A-I and apo A-II concentrations. These results indicate that, in contrast to fibrates, the thiazolidinedione BRL49653 does not have major effects on any of the HDL parameters tested.

In summary, Applicant's data show that BRL49653 treatment decreased serum triglycerides in a dose-dependent fashion without affecting serum total and HDL cholesterol and apo A-I and apo A-II concentrations. The decrease in triglyceride concentrations after BRL49653 treatment was

mainly due to a reduction of the amount of VLDL particles, whose lipid and apolipoprotein composition did not change. BRL49653 treatment did not change triglyceride production in vivo as analyzed by injection of Triton WR-1339. Analysis of the influence of BRL49653 on the expression of LPL and apo C-III, two key-players in triglyceride catabolism, showed a dose-dependent increase in mRNA levels and activity of LPL in epididymal adipose tissue, whereas liver apo C-III mRNA levels remained constant.

10           Without being bound by any theory, Applicant proposes that thiazolidinediones lower plasma triglycerides levels at least in part by inducing the expression of LPL in adipose tissue which increases the removal of triglyceride from plasma.

15           Although thiazolidinediones may influence protein phosphorylation activities as well as cellular  $\text{Ca}^{++}$ -uptake, it is likely that most, if not all, of their actions at the molecular level are mediated via activation of the transcription factor PPAR $\gamma$ /RXR heterodimer. More specifically, BRL49653 has been shown to be a high-affinity synthetic ligand for PPAR $\gamma$ . Furthermore, the adipose-specific PPAR $\gamma$ 2 isoform has been shown to play a crucial role in adipogenesis. PPAR $\gamma$ 2 furthermore regulates the expression of adipose genes, such as the aP2 and phosphoenolpyruvate carboxykinase (PEPCK) genes via a PPRE in its promoter. 20  
25           Similarly, Applicant has identified a PPRE in the human LPL gene promoter, which suggests that the induction of adipose tissue LPL gene expression after BRL49653 is mediated via PPAR $\gamma$ .

In contrast to fenofibrate, treatment with BRL49653 does not increase liver weight. In addition, at doses exceeding those needed to decrease serum triglycerides (up to 20 mg/kg/d during 14d), BRL49653 has no effect on liver apo C-III mRNA levels, nor on the expression of the ACO gene, whose regulation is under strict control of PPAR $\alpha$  (Lee, S. S. T., et al. (1995) Molecular Cellular Biology 15: 3012-3022). These data suggest that, in contrast to fenofibrate, BRL49653 has no or only very little PPAR $\alpha$  activating potential in vivo, and consequently does not provoke peroxisome proliferation in rodents.

In addition, whereas fenofibrate is a very potent hypocholesterolemic drug in rodents, total and HDL cholesterol concentrations remain unchanged upon BRL49653 treatment. These data are in line with previous studies using pioglitazone in the KKAY mouse, a model for Type 2 diabetes, as well as in normal rats. These data indicate that, at least in rat, lowering of serum triglycerides due to increased LPL expression in adipose tissue, does not have major effects on HDL metabolism. Since in rodents serum cholesterol is transported mainly in the HDL fraction, the unchanged serum cholesterol levels after BRL49653 can be explained by the unaltered expression of its major apolipoproteins, apo A-I, apo A-II or apo A-IV, in rat liver. Again, these effects of BRL49653 are in contrast to those of fibrates, which have important effects on the expression of these genes in liver (Staels, B., et al. (1990) Endocrinology 126: 2153-2163; Staels, B., et al. (1992) Arteriosclerosis and Thrombosis 12: 286-294).

A PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist in combination achieved enhanced antidiabetic and cardioprotective effects over either agent alone

Applicant's data show that treatment with the  
5 thiazolidinedione BRL49653 decreases serum triglyceride  
concentrations by enhancing serum triglyceride removal.  
BRL49653 treatment increased lipolysis of triglycerides in  
plasma lipoproteins, leading to an accelerated conversion into  
IDL and LDL particles. Enhanced lipolysis after BRL49653 is  
10 linked to the induction of LPL expression in adipose tissue.  
BRL49653, activates PPAR $\gamma$  while fibrates primarily activate  
the PPAR $\alpha$  isoform and regulate apo C-III and HDL  
apolipoprotein gene expression in liver. Fenofibrate  
treatment does not change the composition of VLDL or IDL+LDL  
15 lipoproteins, suggesting that these drugs have only minor  
effects on VLDL lipolysis. Therefore, although either  
BRL49653 or fenofibrate can reduce plasma triglyceride  
concentrations, they operate through distinct mechanisms.

Such analysis is supported by the observation below  
20 that simultaneous administration of BRL49653 and fenofibrate  
results in more pronounced effects on plasma triglyceride  
profiles than the administration of either agent alone. The  
additive effects of fenofibrate and BRL49653 on the level of  
plasma triglycerides indicate that drugs which combine PPAR $\alpha$   
25 and  $\gamma$  activation would be extremely efficient triglyceride-  
lowering agents, and would be useful in the treatment of  
different forms of hypertriglyceridemia, such as found in Type  
2 diabetes and familial combined hypertriglyceridemia. Lower  
doses of drugs can be used to limit side-effects such as

increased liver (seen after fenofibrate treatment) and adipose tissue (seen after BRL49653 treatment) weights without compromising their therapeutic efficacy.

Pharmacologic agents that lower plasma glucose, insulin, triglyceride, uric acid, and fibrinogen levels, blood pressure or body weight would reduce the risk of cardiovascular disease. Similarly, pharmacologic agents that raise HDLc levels and reduce the appearance of small dense LDLc in plasma would decrease the risk of cardiovascular disease.

***Simultaneous treatment with a PPAR $\alpha$  agonist and a PPAR $\gamma$  agonist have combined effects on triglyceride levels***

Two animal models were used to test Applicant's theory that coactivation of PPAR $\gamma$  and PPAR $\alpha$  is useful in the treatment of Type 2 diabetes and cardiovascular disease with diabetic or pre-diabetic conditions.

The rat was used to demonstrate the activities of triglyceride-lowering compounds by monitoring the levels of plasma triglycerides and the distribution of triglycerides in lipoproteins particles such as Very Low Density Lipoproteins (VLDL)). The db/db mouse was employed as a model of Type 2 diabetes. This animal has a genetic defect in the cellular receptor for the hormone leptin. Like human diabetic patients, db/db mice show elevated levels of glucose and triglycerides. Db/db mice respond to antidiabetic agents such as thiazolidinediones.

Figure 1 is a graph showing the levels of plasma triglyceride in db/db diabetic mice treated for 14 days with a

PPAR $\alpha$  agonist (gemfibrozil), a PPAR $\gamma$  agonist (BRL 49653), or the agents in combination. Total plasma triglyceride levels were determined on the days indicated. At suboptimal doses of 50 mg/kg/day for gemfibrozil or 0.4 mg/kg/day for BRL 49653, neither agent was very effective in lowering triglyceride levels. The same doses administered in combination showed significant triglyceride lowering effects. This shows that the actions of a PPAR $\alpha$  agonist and a PPAR $\gamma$  agonist can be synergistic, thus allowing for lower therapeutic doses of each compound for improved safety and cost profiles. In addition, with optimal amounts of a PPAR $\alpha$  agonist and a PPAR $\gamma$  agonist, the level of triglyceride can be lowered to an extent greater than with either compound alone. Such synergistic effects can also be used to lower elevated levels of insulin, fibrinogen, PAI-1, and LDL, etc.

Figure 2 is a graph showing the distribution of triglyceride levels within the plasma lipoproteins of normal chow-fed rats treated for 14 days with a PPAR $\alpha$  agonist (fenofibric acid), a PPAR $\gamma$  agonist (thiazolidinedione compound BRL 49653), or the agents in combination. Lipoproteins were isolated from rat plasma by ultracentrifugation, and separated according to hydrated radius on an agarose gel column. Triglyceride levels were determined on column fractions. Either BRL 49653 (Figure 2B) or fenofibrate (Figure 2C) alone lowered triglyceride levels. The combination of agents showed enhanced triglyceride lowering activity (Figure 2D). In this study, BRL 49653 was administered at 10 mg/kg/day, which is higher than its maximum effect dose for this model. Fenofibrate was administered at 40 mg/kg/day, which is at

least equal to its maximum effect dose in this model. The combination of both agents at maximum effect doses showed enhanced activity over that of either agent alone.

5        ***Simultaneous treatment with BRL49653 and fenofibrate have combined effects on liver apolipoprotein and adipose tissue LPL gene expression***

The effects of combined treatment with BRL49653 and fenofibrate on liver apolipoprotein gene expression were  
10        investigated next. Treatment with BRL49653 alone did not affect the expression of any of the apolipoproteins analyzed in liver, e.g. apo C-III, whereas LPL mRNA levels increased to a comparable extent (2-fold). In contrast, fenofibrate treatment alone provoked significant decreases in liver apo A-  
15        I, apo A-II, apo A-IV and apo C-III mRNA levels without changing adipose tissue LPL mRNA levels.

Treatment with fenofibrate and BRL49653 together resulted in a similar decrease in liver apolipoprotein mRNA levels and increase in adipose tissue LPL mRNA levels (2-fold)  
20        as treatment with fenofibrate and BRL49653 alone respectively.

Finally, the effects of BRL49653 treatment were investigated on the expression of the acyl CoA oxidase (ACO) gene, whose regulation by fibrates is under the control of the PPAR $\alpha$  isoform. Treatment with fenofibrate, either alone or in  
25        combination with BRL49653, resulted in more than 10-fold induction of liver ACO mRNA levels, confirming previous observations in rats. In contrast, administration of BRL49653 did not change liver ACO mRNA levels, even when given for 14 days at doses of 10 and 20 mg/kg/d (Control: 100%  $\pm$  32;

BRL49653 10 mg/kg/d: 103%  $\pm$  46; BRL49653 20 mg/kg/d: 86%  $\pm$  39). These data show that treatment with BRL49653 does not result in any significant activation of PPAR $\alpha$  in the liver, even at doses higher than required to lower serum triglycerides, indicating that the triglyceride lowering activity of BRL49653 occurs independent of PPAR $\alpha$  activation.

In summary, simultaneous administration of fenofibrate and BRL49653 resulted both in a decrease in liver apo C-III and an increase in adipose tissue LPL mRNA levels and a more pronounced decrease in serum triglyceride-rich lipoprotein concentrations than achieved by either agent alone. These data suggest that drugs activating both PPAR $\alpha$  and  $\gamma$  would be more effective hypotriglyceridemic drugs.

Accordingly, this invention discloses that a pharmaceutical composition containing effective amounts of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist may be utilized in the treatment of Type 2 diabetes and cardiovascular disease with diabetic or pre-diabetic conditions. PPAR $\gamma$  agonists act as insulin sensitizers or insulin mimetics to lower plasma levels of glucose, insulin and triglycerides while PPAR $\alpha$  agonists normalize lipid and coagulation factor metabolism by elevating plasma levels of HDLc, and lowering plasma levels of triglycerides, small dense LDL, fibrinogen, and PAI-1.

## MATERIALS AND METHODS

### Materials

BRL49653 was synthesized at Ligand Pharmaceuticals. Fenofibrate and Triton WR-1339 (Tyloxapol<sup>®</sup>) were obtained from Sigma (St. Louis, MO) and carboxymethylcellulose from Serva



(Heidelberg, Germany). GW2331 was synthesized at Ligand Pharmaceuticals Inc. Gemfibrozil was obtained from Sigma.

#### **Animals**

5           Male Sprague-Dawley rats were randomized to treatment groups and treated daily intragastrically for the indicated periods of time with BRL49653 and/or fenofibrate suspended in 1% carboxymethylcellulose at the indicated doses. Control animals received an equal volume (5 ml/kg/d) of  
10   carboxymethylcellulose solution. At the end of the experiments animals were weighed and sacrificed by exsanguination while under ether anesthesia. Blood was collected and serum was separated and used within 1 week for analysis of lipids, lipoproteins and apolipoproteins. Liver  
15   and epididymal fat pads were removed immediately, weighed and frozen in liquid nitrogen.

Female diabetic C57BLKS/J-m+/+db mice (47 days old at commencement of study) were dosed with vehicle (0.9% carboxymethyl cellulose, 9.95% polyethylene glycol and 0.05%  
20   Tween 80), or drugs (as indicated) with 0.6 ml per 42 g, by gavage once a day. Blood was drawn after a 3 hour fast on days indicated.

#### **Serum glucose, lipid, apolipoprotein and lipoprotein measurements**

25           Serum glucose and lipoprotein lipid concentrations (total and free cholesterol, triglycerides, phospholipids) were measured colorimetrically using enzymatic test kits from Boehringer Mannheim (Mannheim, Germany). Serum HDL

cholesterol content was determined after precipitation of apo B-containing lipoproteins with phosphotungstic acid/Mg (Boehringer Mannheim). Serum levels of rat apo A-I and apo A-II were measured by an immunonephelometric assay using  
5 specific polyclonal antibodies.

Plasma insulin was measured by Linco insulin radioimmunoassay against rat insulin antibody (Linco Research, St. Louis MO).

The lipoprotein fractions (VLDL,  $d < 1.006$ ; IDL+LDL,  $d = 1.006-1.063$ ; HDL,  $d = 1.063-1.21$  g/ml) were isolated by  
10 sequential ultracentrifugation of pooled rat serum (Havel, R. J., et al. (1955) J. Clin. Invest. 34: 1345-1353). Each fraction was further purified by a second ultracentrifugation at the same density intervals before analysis. After  
15 extensive dialysis at 4°C against 10mM phosphate-buffered saline (PBS) at pH 7.2 containing 10  $\mu$ M EDTA, the protein concentration of each lipoprotein fraction was determined by the method of Lowry et al. (1951 J. Biol. Chem. 193: 265-275).

For fast protein liquid chromatography (FPLC) size  
20 fractionation of lipoproteins, 300  $\mu$ g of serum lipoprotein protein ( $d < 1.21$  g/ml) isolated from individual rats was injected on a Sepharose 6HR 10/30 prepacked column (Pharmacia, Uppsala, Sweden) and eluted at a constant flow rate of 0.2 ml/min with PBS pH 7.2. The effluent was monitored at 280 nm,  
25 collected in 0.3 ml fractions and cholesterol and triglyceride concentrations determined in 0.1 ml of each fraction.

The distribution of lipoproteins in serum from individual rats was analyzed by non-denaturing discontinuous gradient polyacrylamide gel electrophoresis (Lipofilm kit,

Sebia, Issy-les-Moulineaux, France). Briefly, 5  $\mu$ l of Sudan Black prestained samples were electrophoresed at 10°C for 45 min at a constant voltage of 170V in a Tris-barbital buffer, pH 8.3. The wet gels were immediately scanned using the Biorad Gel-Doc 1000 system.

The apolipoprotein composition of isolated lipoproteins was analyzed by non-reducing SDS-PAGE as described (Tailleux, A., et al. (1993) J. Lipid Res. 34: 719-728). Protein samples (15  $\mu$ g) were heat-denatured and loaded on 3-19% gels, separated by electrophoresis at 150V for 45 min and visualized by Coomassie brilliant blue staining. The distribution of the apo C-II and apo C-III subspecies was analyzed by isoelectric focusing gel electrophoresis. VLDL proteins (200  $\mu$ g) were precipitated with a mixture of acetone and ethanol (1:1) and delipidated with diethylether at -20°C (Warnick, G. R., et al. (1979) Clinical Chemistry 25: 279-284). The delipidated proteins were then electrophoresed on a polyacrylamide gel at 4°C for 30 min at 100V, 14 h at 250V and 1 h at 1000V. Gel preparation, fixing and protein staining were performed as described (Kane, J. P. (1973) Analytical Biochemistry 53: 350-364.).

#### *Determination of in vivo triglyceride synthesis*

Rats (n=3/group) were treated for 7 days with BRL49653 (10 mg/kg/d) or vehicle. At the end of the treatment period rats were injected in the caudal vein with a 20% w/v Triton WR-1339 solution at 500 mg/kg of body weight. Blood was collected under ether anesthesia just prior to injection

and at 1 and 2 h after injection. Serum triglycerides were determined subsequently.

#### **Measurement of adipose tissue LPL activity**

5 LPL was measured in extracts from epididymal adipose tissue according to the procedure of Ramirez, I., et al. (1985) Biochem. J. 232: 229-236. One unit of enzyme activity was defined as the amount of enzyme which releases 1  $\mu$ mole oleate/min at 25°C.

#### **RNA analysis**

10 RNA was isolated from liver and epididymal adipose tissue by the acid guanidinium thiocyanate/phenol/chloroform method (Chomczynski, P. and N. Sacchi (1987) Analytical  
15 Biochemistry 162: 156-159). Northern and dot blot analysis of total cellular RNA was performed as described (Staels, B., et al. (1992) Arteriosclerosis and Thrombosis 12: 286-294). Rat apo A-I, apo A-II, apo A-IV, apo C-III and human LPL cDNA clones were used as probes (Staels, B., et al. (1992)  
20 Arteriosclerosis and Thrombosis 12: 286-294; Auwerx, J., et al. (1988) Biochemistry 27: 2651-2655; Staels, B., et al. (1995) J. Clin. Invest. 95: 705-712). cDNA clones for  $\beta$ -actin (Cleveland, D. W., et al. (1980) Cell 20: 95-105) and 36B4 (Masiakowski, P., et al. (1982) Nucleic Acids Research 10:)  
25 (encoding the human acidic ribosomal phosphoprotein PO (Laborda, J. (1991) Nucleic Acids Research 19: 3998)) were used as control probes. All probes were labeled by random primed labeling (Boehringer Mannheim). Filters were hybridized to  $1.5 \times 10^6$  cpm/ml of each probe as described

(Staels, B., et al. (1992) Arteriosclerosis and Thrombosis 12: 286-294). They were washed once in 500 ml 75 mM NaCl, 7.5 mM sodium citrate and 0.1% SDS for 10' at room temperature and twice for 30' at 65°C and subsequently exposed to X-ray film (X-OMAT-AR, Kodak). Autoradiograms were analyzed by quantitative scanning densitometry (Biorad GS670 Densitometer) as described (Staels, B., et al. (1992) Arteriosclerosis and Thrombosis 12: 286-294).

#### 10 Pharmaceutical Formulations and Modes of Administration

The particular compounds that affect the disorders or conditions of interest can be administered to a patient either by themselves, or in pharmaceutical compositions where they are mixed with suitable carriers or excipient(s). In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of a agent or agents such as these is administered. A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient.

The compounds also can be prepared as pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include acid addition salts such as those containing hydrochloride, sulfate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, cyclohexylsulfamate and quinate. (See e.g., PCT/US92/03736). Such salts can be derived using acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic

acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid.

5           Pharmaceutically acceptable salts can be prepared by standard techniques. For example, the free base form of the compound is first dissolved in a suitable solvent such as an aqueous or aqueous-alcohol solution, containing the appropriate acid. The salt is then isolated by evaporating  
10 the solution. In another example, the salt is prepared by reacting the free base and acid in an organic solvent.

          Carriers or excipients can be used to facilitate administration of the compound, for example, to increase the solubility of the compound. Examples of carriers and  
15 excipients include calcium carbonate, calcium phosphate, various sugars or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents.

          Toxicity and therapeutic efficacy of such compounds  
20 can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the  
25 therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies

preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. Levels in plasma  
5 may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl et al., in The Pharmacological Basis of Therapeutics, 1975, Ch. 1 p. 1). It  
10 should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate  
15 (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic  
20 evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

Depending on the specific conditions being treated,  
25 such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or

intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just  
5 to name a few.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such  
10 transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of  
15 the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered  
20 parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules,  
25 liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be



encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Liposomes may be used for encapsulated delivery.

Pharmaceutical formulations disclosed or described in Boehm, et al., WO94/15902 are incorporated by reference herein.

All publications referenced are incorporated by reference herein, including the nucleic acid sequences and amino acid sequences listed in each publication. All the compounds disclosed and referred to in the publications mentioned above are incorporated by reference herein,

including those compounds disclosed and referred to in articles cited by the publications mentioned above.

This application is converted from provisional application serial no. 60/022,949, which is incorporated by  
5 reference herein in its entirety, including its figures, formulas and amino acid sequences and nucleic acid sequences described or disclosed therein.

Other embodiments of this invention are disclosed in the following claims.

Table 1. Influence of BRL49653 on body, liver and fat pad weights in rats

5	Dose (mg/kg/ d)	Body weight (g)	Liver weight (%)	Adipose tissue weight (%)
10	0 1 2 5	$344 \pm 22^a$ $355 \pm 21^a$ $361 \pm 18^a$ $338 \pm 9^a$	$4.9 \pm 0.2^a$ $5.1 \pm 0.2^a$ $5.3 \pm 0.2^a$ $5.1 \pm 0.4^a$	$0.8 \pm 0.1^c$ $0.9 \pm 0.0^{bc}$ $1.0 \pm 0.1^{ab}$ $1.2 \pm 0.2^a$

15

Normal adult male rats (n=4/group) received BRL49653 intragastrically at the indicated dose for 7 days. Body, liver and epididymal adipose tissue weights were recorded at the end of the treatment period. Statistically significant differences (ANOVA;  $p < 0.05$ ) are observed between values followed by different letters.

20

Table 2. Influence of BRL49653 on serum lipid and glucose concentrations in rats

	Dose (mg/kg /d)	Total choles. (mg/dl)	Free choles. (mg/dl)	HDL choles. (mg/dl)	Triglyce rides (mg/dl)	Glucose (mg/dl)
5						
10	0	81 ± 5 <sup>a</sup>	17 ± 1 <sup>a</sup>	69 ± 8 <sup>a</sup>	190±51 <sup>a</sup>	157±16 <sup>a</sup>
	1	80 ± 13 <sup>a</sup>	18 ± 3 <sup>a</sup>	68±11 <sup>a</sup>	161±50 <sup>a</sup>	151±11 <sup>a</sup>
	2	74 ± 15 <sup>a</sup>	18 ± 3 <sup>a</sup>	67±16 <sup>a</sup>	138±48 <sup>ab</sup>	177±18 <sup>a</sup>
	5	86 ± 10 <sup>a</sup>	20 ± 2 <sup>a</sup>	73±11 <sup>a</sup>	75 ± 23 <sup>b</sup>	185±13 <sup>a</sup>

15

Normal adult male rats (n=4/group) received BRL49653 intragastrically at the indicated dose for 7 days. Serum total, free and HDL cholesterol, triglyceride and glucose concentrations were measured as described in Methods.

20 Statistically significant differences (ANOVA; p<0.05) are observed between values followed by different letters.

Table 3. Influence of BRL49653 on lipoprotein composition in rats

5	VLDL (d<1.006)	Control	BRL49653 5 mg/kg/d	BRL49653 10 mg/kg/d	BRL49653 20 mg/kg/d
10	Total cholesterol (%)	5.5	5.2	5.8	7.8
	Triglyceride (%)	74.0	76.4	74.0	70.0
	Phospholipid (%)	12.0	11.3	13.2	13.1
15	Protein (%)	8.5	7.0	7.0	9.5
20	IDL + LDL 1.006<d<1.063	Control	BRL49653 5 mg/kg/d	BRL49653 10 mg/kg/d	BRL49653 20 mg/kg/d
25	Total cholesterol (%)	19.4	22.5	19.3	25.3
	Triglyceride (%)	42.0	37.0	35.4	28.7
	Phospholipid (%)	15.8	17.4	15.9	20.7
30	Protein (%)	22.8	23.1	29.4	25.3

	HDL (1.063<d<1.21)	Control	BRL49653 5 mg/kg/d	BRL49653 10 mg/kg/d	BRL49653 20 mg/kg/d
5	Total cholesterol (%)	28.8	30.0	28.0	29.7
	Triglyceride (%)	2.9	2.5	1.8	1.5
10	Phospholipid (%)	25.0	23.9	23.8	27.0
	Protein (%)	43.3	43.6	46.4	41.8

15 Normal adult male rats (n=4/group) were treated  
intragastrically with BRL49653 at the indicated doses daily  
for 14 days. Serum lipoproteins were isolated by  
sequential density ultracentrifugation at the indicated  
20 density intervals and lipoprotein total cholesterol,  
triglyceride, phospholipid and protein concentrations were  
measured and expressed as percentage of total as described  
in Methods.



What is Claimed is:

1. A method for treating Type 2 diabetes or cardiovascular disease with diabetic or pre-diabetic conditions in a host suffering therefrom, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.
2. The method of claim 1, wherein said PPAR $\gamma$  agonist and said PPAR $\alpha$  agonist are administered as a pharmaceutical composition comprising said PPAR $\gamma$  agonist and said PPAR $\alpha$  agonist with a pharmaceutically acceptable carrier or excipient.
3. The method of claim 1, wherein said PPAR $\gamma$  agonist and said PPAR $\alpha$  agonist are administered as a single dosage unit.
4. The method of claim 1, wherein said PPAR $\gamma$  agonist and said PPAR $\alpha$  agonist are the same compound.
5. The method of claim 4, wherein said compound is 2-(4-[2,4-Difluorophenyl]-1-heptylureido)ethylphenoxy)-2-methylbutyric acid or a pharmaceutically acceptable salt thereof.
6. The method of claim 1, wherein said PPAR $\gamma$  agonist is a thiazolidinedione compound or a pharmaceutically acceptable salt thereof.

7. The method of claim 6, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075, 5 darglitazone, and analogs, derivatives, and congeners of the above listed compounds.

8. The method of claim 1, further comprising the step of administering to said host a pharmaceutically 10 effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizers, insulin mimetic, metformin, acarbose, or sulfonyl ureas.

9. The method of claim 1, wherein said PPAR $\gamma$  agonist 15 and said PPAR $\alpha$  agonist are orally, topically, intravenously, transdermally, suppositorily or parentally administered.

10. The method of claim 1, wherein said PPAR $\alpha$  agonist 20 is selected from the group consisting of gemfibrozil fenofibrate, bezafibrate, clofibrate, ciprofibrate, and analogs, derivatives, congeners, and pharmaceutically acceptable salts thereof.

25 11. The method of claim 1, further comprising the step of administering to said host a pharmaceutically effective amount of an RXR agonist.

12. The method of claim 1, further comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\beta$  or NUC1 modulator which reduces or relieves the repression of PPAR $\alpha$  or PPAR $\gamma$  by PPAR $\beta$  or NUC1.

13. A method for treating Type 2 diabetes or cardiovascular disease with diabetic or pre-diabetic conditions in a host suffering therefrom, comprising the steps of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist, and administering to said host a pharmaceutically effective amount of a PPAR $\alpha$  agonist.

14. A pharmaceutical composition adapted for the treatment of Type 2 diabetes or cardiovascular disease with diabetic or pre-diabetic conditions in a host suffering therefrom comprising

(a) a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist; and

(b) a pharmaceutically acceptable carrier.

15. The composition of claim 14, wherein said PPAR $\gamma$  agonist is a thiazolidinedione compound or a pharmaceutically acceptable salt thereof.

16. The composition of claim 15, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone,

ciglitazone, WAY-120,744, englitazone, AD 5075, darglitazone, and analogs, derivatives, and congeners of the above listed compounds.

5           17. The composition of claim 14 further comprising a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer, insulin mimetic, metformin, acarbose, or sulfonyl ureas.

10           18. The composition of claim 14, wherein said PPAR $\alpha$  agonist is selected from the group consisting of gemfibrozil fenofibrate, bezofibrate, clofibrate, ciprofibrate, and analogs, derivatives, congeners, and pharmaceutically acceptable salts thereof.

15           19. The composition of claim 14 further comprising a pharmaceutically effective amount of an RXR agonist.

20           20. The composition of claim 14 further comprising a pharmaceutically effective amount of a PPAR $\beta$  or NUC1 modulator which reduces or relieves the repression of PPAR $\alpha$  or PPAR $\gamma$  by PPAR $\beta$  or NUC1.

25           21. A method for treating hypertriglyceridemia in a host suffering therefrom, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

22. A method for treating hyperglycemia in a host suffering therefrom, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

5

23. A method for treating hyperinsulinemia in a host suffering therefrom, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

10

24. A method for treating hyperfibrinogenemia in a host suffering therefrom, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

15

25. A method for treating hypertension in a host suffering therefrom, comprising, the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

20

26. A method for treating obesity in a host suffering therefrom, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

25

27. A method for treating syndrome X in a host suffering therefrom, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

28. A method for increasing the level of HDL cholesterol in a host, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

5

29. A method for increasing insulin sensitivity in a host, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

10

30. A method for increasing glucose uptake in the adipose or muscle tissue of a host, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

15

31. A method for preventing Type 2 diabetes or cardiovascular disease in a pre-diabetic host, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

20

32. A method for preventing insulin resistance in a host, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

25

33. A method for preventing hyperglycemia in a host, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

34. A method for preventing hyperinsulinemia in a host, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

5

35. A method for preventing hypertriglyceridemia in a host, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

10

36. A method for preventing hyperfibrinogenemia in a host, comprising the step of administering to said host a pharmaceutically effective amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist.

15

37. A method for lowering the level of plasma triglyceride in a mammal, comprising the step of administering to said mammal an amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist effective to lower said level of plasma triglyceride.

20

38. A method for lowering the level of plasma glucose in a mammal, comprising the step of administering to said mammal an amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist effective to lower said level of plasma glucose.

25

39. A method for lowering the level of plasma insulin in a mammal, comprising the step of administering to said

mammal an amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist effective to lower said level of plasma insulin.

40. A method for lowering the body weight of a  
5 mammal, comprising the step of administering to said mammal an amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist effective to lower said body weight.

41. A method for lowering the blood pressure of a  
10 mammal, comprising the step of administering to said mammal an amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist effective to lower said blood pressure.

42. A method for lowering the level of plasma  
15 fibrinogen in a mammal, comprising the step of administering to said mammal an amount of a PPAR $\gamma$  agonist and a PPAR $\alpha$  agonist effective to lower said level of plasma fibrinogen.

20 43. A method of preparing a compound for treating Type 2 diabetes or cardiovascular disease with diabetic or pre-diabetic conditions, comprising the steps of:

- (A) providing a plurality of candidate compounds;
- (B) bringing each of said plurality of candidate  
25 compounds into contact with a PPAR $\gamma$  in vivo or in vitro and detecting its ability to stimulate or increase the activity of said PPAR $\gamma$  or its ability to bind to said PPAR $\gamma$ ;



(C) bringing each of said plurality of candidate compounds into contact with a PPAR $\alpha$  *in vivo* or *in vitro* and detecting its ability to stimulate or increase the activity of said PPAR $\alpha$  or its ability to bind to said PPAR $\alpha$ ; and

5 (D) selecting a candidate compound which stimulates or increases the activity of both said PPAR $\gamma$  and PPAR $\alpha$  or binds both said PPAR $\gamma$  and PPAR $\alpha$  and packaging a pharmaceutically effective amount of said candidate compound for treating Type 2 diabetes or cardiovascular  
10 disease with diabetic or pre-diabetic conditions.

44. A method of preparing a compound for treating hypertriglyceridemia, comprising the steps of:

(A) providing a plurality of candidate compounds;

15 (B) bringing each of said plurality of candidate compounds into contact with a PPAR $\gamma$  *in vivo* or *in vitro* and detecting its ability to stimulate or increase the activity of said PPAR $\gamma$  or its ability to bind to said PPAR $\gamma$ ;

(C) bringing each of said plurality of candidate  
20 compounds into contact with a PPAR $\alpha$  *in vivo* or *in vitro* and detecting its ability to stimulate or increase the activity of said PPAR $\alpha$  or its ability to bind to said PPAR $\alpha$ ; and

(D) selecting a candidate compound which stimulates or increases the activity of both said PPAR $\gamma$  and PPAR $\alpha$  or  
25 binds both said PPAR $\gamma$  and PPAR $\alpha$  and packaging a pharmaceutically effective amount of said candidate compound for treating hypertriglyceridemia.

45. A method of preparing a compound for treating hyperglycemia, comprising the steps of:

(A) providing a plurality of candidate compounds;

5 (B) bringing each of said plurality of candidate compounds into contact with a PPAR $\gamma$  in vivo or in vitro and detecting its ability to stimulate or increase the activity of said PPAR $\gamma$  or its ability to bind to said PPAR $\gamma$ ;

10 (C) bringing each of said plurality of candidate compounds into contact with a PPAR $\alpha$  in vivo or in vitro and detecting its ability to stimulate or increase the activity of said PPAR $\alpha$  or its ability to bind to said PPAR $\alpha$ ; and

15 (D) selecting a candidate compound which stimulates or increases the activity of both said PPAR $\gamma$  and PPAR $\alpha$  or binds both said PPAR $\gamma$  and PPAR $\alpha$  and packaging a pharmaceutically effective amount of said candidate compound for treating hyperglycemia.

46. A method of preparing a compound for treating hyperinsulinemia, comprising the steps of:

20 (A) providing a plurality of candidate compounds;

(B) bringing each of said plurality of candidate compounds into contact with a PPAR $\gamma$  in vivo or in vitro and detecting its ability to stimulate or increase the activity of said PPAR $\gamma$  or its ability to bind to said PPAR $\gamma$ ;

25 (C) bringing each of said plurality of candidate compounds into contact with a PPAR $\alpha$  in vivo or in vitro and detecting its ability to stimulate or increase the activity of said PPAR $\alpha$  or its ability to bind to said PPAR $\alpha$ ; and

(D) selecting a candidate compound which stimulates or increases the activity of both said PPAR $\gamma$  and PPAR $\alpha$  or binds both said PPAR $\gamma$  and PPAR $\alpha$  and packaging a pharmaceutically effective amount of said candidate  
5 compound for treating hyperinsulinemia.

47. A method of preparing a compound for treating hyperfibrinogenemia, comprising the steps of:

- (A) providing a plurality of candidate compounds;  
10 (B) bringing each of said plurality of candidate compounds into contact with a PPAR $\gamma$  *in vivo* or *in vitro* and detecting its ability to stimulate or increase the activity of said PPAR $\gamma$  or its ability to bind to said PPAR $\gamma$ ;  
(C) bringing each of said plurality of candidate  
15 compounds into contact with a PPAR $\alpha$  *in vivo* or *in vitro* and detecting its ability to stimulate or increase the activity of said PPAR $\alpha$  or its ability to bind to said PPAR $\alpha$ ; and  
(D) selecting a candidate compound which stimulates or increases the activity of both said PPAR $\gamma$  and PPAR $\alpha$  or  
20 binds both said PPAR $\gamma$  and PPAR $\alpha$  and packaging a pharmaceutically effective amount of said candidate compound for treating hyperfibrinogenemia.

48. A method of preparing a compound for treating  
25 hypertension, comprising the steps of:

- (A) providing a plurality of candidate compounds;  
(B) bringing each of said plurality of candidate compounds into contact with a PPAR $\gamma$  *in vivo* or *in vitro* and

detecting its ability to stimulate or increase the activity of said PPAR $\gamma$  or its ability to bind to said PPAR $\gamma$ ;

(C) bringing each of said plurality of candidate compounds into contact with a PPAR $\alpha$  *in vivo* or *in vitro* and  
5 detecting its ability to stimulate or increase the activity of said PPAR $\alpha$  or its ability to bind to said PPAR $\alpha$ ; and

(D) selecting a candidate compound which stimulates or increases the activity of both said PPAR $\gamma$  and PPAR $\alpha$  or binds both said PPAR $\gamma$  and PPAR $\alpha$  and packaging a  
10 pharmaceutically effective amount of said candidate compound for treating hypertension.

49. A method of preparing a compound for treating obesity, comprising the steps of:

15 (A) providing a plurality of candidate compounds;

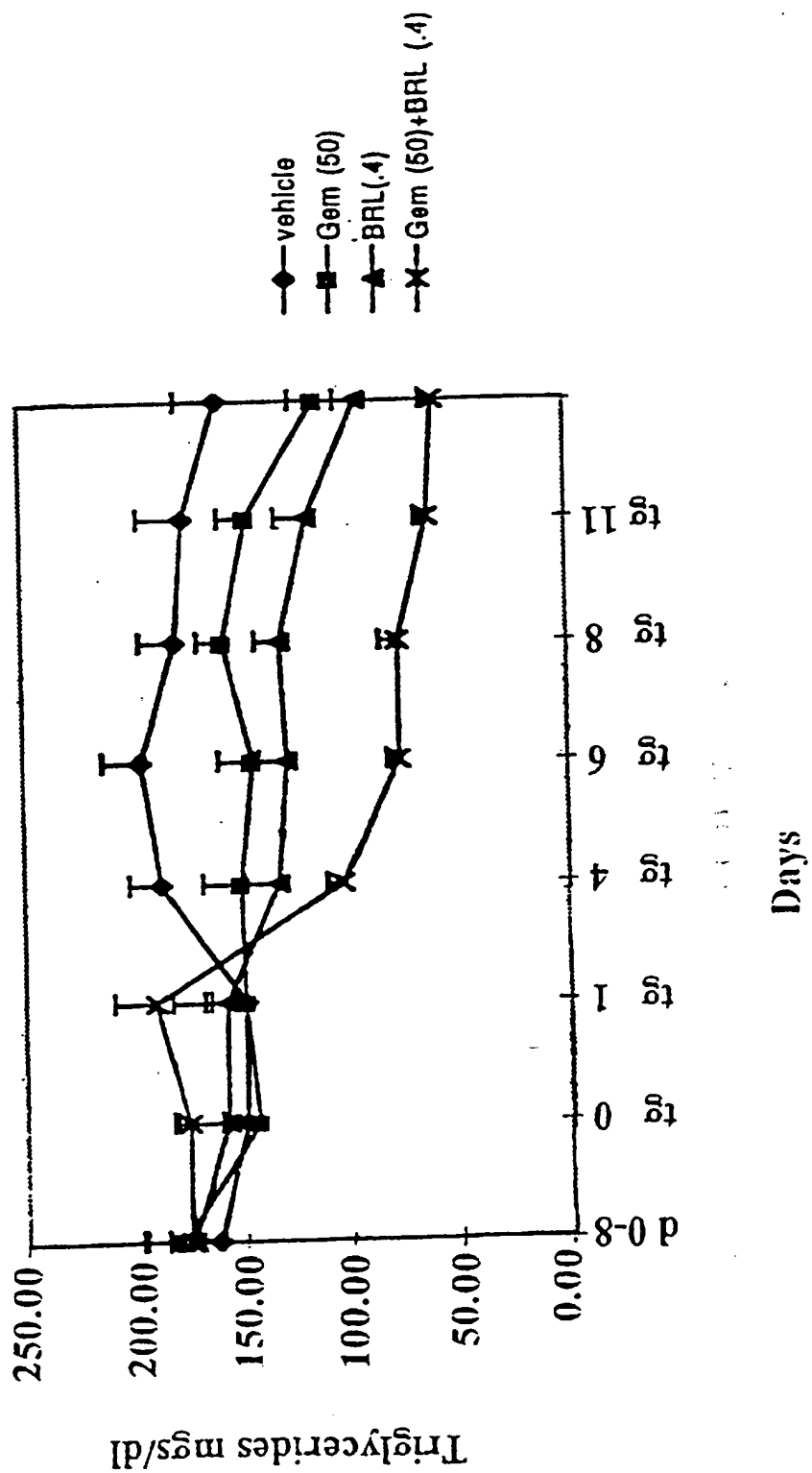
(B) bringing each of said plurality of candidate compounds into contact with a PPAR $\gamma$  *in vivo* or *in vitro* and detecting its ability to stimulate or increase the activity of said PPAR $\gamma$  or its ability to bind to said PPAR $\gamma$ ;

20 (C) bringing each of said plurality of candidate compounds into contact with a PPAR $\alpha$  *in vivo* or *in vitro* and detecting its ability to stimulate or increase the activity of said PPAR $\alpha$  or its ability to bind to said PPAR $\alpha$ ; and

(D) selecting a candidate compound which stimulates or  
25 increases the activity of both said PPAR $\gamma$  and PPAR $\alpha$  or binds both said PPAR $\gamma$  and PPAR $\alpha$  and packaging a pharmaceutically effective amount of said candidate compound for treating obesity.

Figure 1

Triglyceride levels in db/db mice



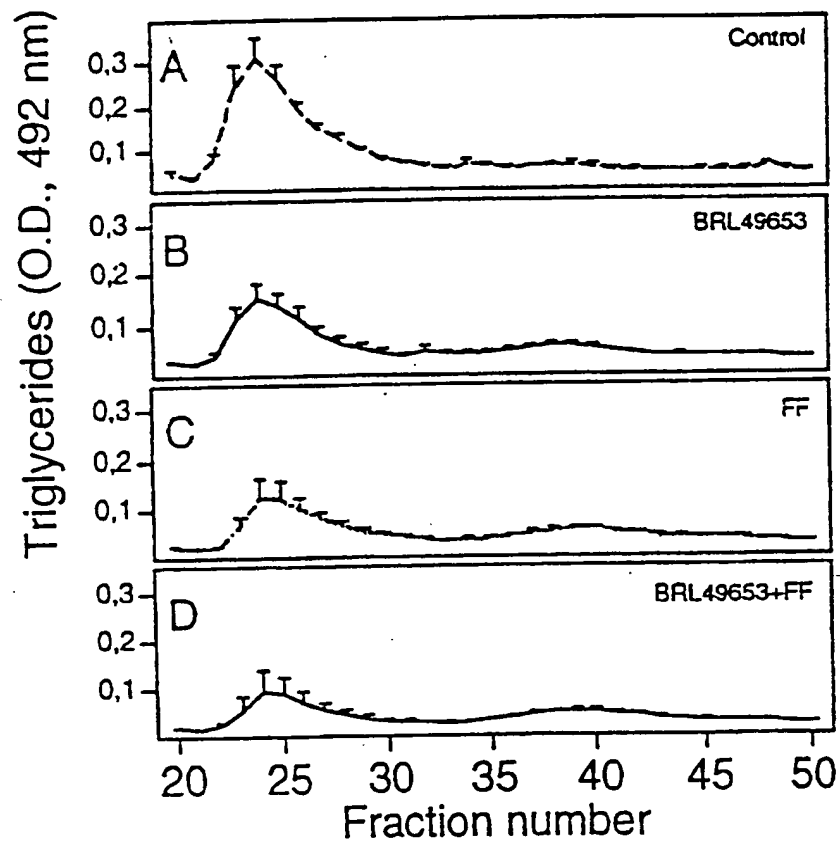


Figure 2

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>A61K 31/425, 31/19 //</b> <b>(A61K 31/425, 31:19)</b>		<b>A3</b>	(11) International Publication Number: <b>WO 98/05331</b> (43) International Publication Date: 12 February 1998 (12.02.98)																																								
(21) International Application Number: <b>PCT/US97/13605</b> (22) International Filing Date: 1 August 1997 (01.08.97) (30) Priority Data: 60/022,949 2 August 1996 (02.08.96) <b>US</b> (71) Applicant: <b>LIGAND PHARMACEUTICALS INCORPORATED [US/US]; 9393 Towne Centre Drive, San Diego, CA 92121 (US).</b> (72) Inventors: <b>PATERNITI, James, R.; 10833 Birch Bluff Avenue, San Diego, CA 92131 (US). BRIGGS, Michael, R.; 12156 Charbono Street, San Diego, CA 92131 (US). MUKHERJEE, Ranjan; 11341 Avenida De Los Lobos, San Diego, CA 92127 (US). AUWERX, Johan; 60, route de Hasnon, F-59178 Millionfosse (FR). STAEL, Bart; 155, D'Huartlaan, B-1500 Kraainem (BE).</b> (74) Agents: <b>CHEN, Anthony, C. et al.; Lyon &amp; Lyon LLP, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).</b>		(81) Designated States: <b>AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</b>  <b>Published</b> <i>With international search report.</i> (88) Date of publication of the international search report: 7 May 1998 (07.05.98)																																									
(54) Title: <b>PREVENTION OR TREATMENT OF TYPE 2 DIABETES OR CARDIOVASCULAR DISEASE WITH PPAR MODULATORS</b>																																											
<p style="text-align: center;"><b>Triglyceride levels in db/db mice</b></p> <table border="1"><caption>Estimated Triglyceride levels (mg/dl) from graph</caption><thead><tr><th>Days</th><th>vehicle</th><th>Gem (50)</th><th>BRL(4)</th><th>Gem (50)+BRL (4)</th></tr></thead><tbody><tr><td>d-8</td><td>175</td><td>175</td><td>175</td><td>175</td></tr><tr><td>t0</td><td>175</td><td>175</td><td>175</td><td>175</td></tr><tr><td>t1</td><td>190</td><td>150</td><td>150</td><td>150</td></tr><tr><td>t4</td><td>190</td><td>150</td><td>130</td><td>75</td></tr><tr><td>t6</td><td>200</td><td>150</td><td>125</td><td>75</td></tr><tr><td>t8</td><td>180</td><td>150</td><td>125</td><td>75</td></tr><tr><td>t11</td><td>170</td><td>150</td><td>125</td><td>75</td></tr></tbody></table>				Days	vehicle	Gem (50)	BRL(4)	Gem (50)+BRL (4)	d-8	175	175	175	175	t0	175	175	175	175	t1	190	150	150	150	t4	190	150	130	75	t6	200	150	125	75	t8	180	150	125	75	t11	170	150	125	75
Days	vehicle	Gem (50)	BRL(4)	Gem (50)+BRL (4)																																							
d-8	175	175	175	175																																							
t0	175	175	175	175																																							
t1	190	150	150	150																																							
t4	190	150	130	75																																							
t6	200	150	125	75																																							
t8	180	150	125	75																																							
t11	170	150	125	75																																							
(57) Abstract <p>This invention relates to methods and compositions for the prevention and treatment of Type 2 diabetes and cardiovascular disease with diabetic or pre-diabetic conditions or symptoms associated therewith using both a PPAR<math>\gamma</math> agonist and a PPAR<math>\alpha</math> agonist or a compound which activates both PPAR<math>\gamma</math> and PPAR<math>\alpha</math>. A preferred PPAR<math>\gamma</math> agonist is a thiazolidinedione compound, including BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075, darglitazone, and congeners, analogs, derivatives and pharmaceutically acceptable salts thereof. A preferred PPAR<math>\alpha</math> agonist is a fibrate compound including gemfibrozil, fenofibrate, bezofibrate, clofibrate, ciprofibrate, and analogs, derivatives and pharmaceutically acceptable salts thereof.</p>																																											

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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 97/13605

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/425 A61K31/19

//(A61K31/425,31:19)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WILLSON T M ET AL: "THE STRUCTURE-ACTIVITY RELATIONSHIP BETWEEN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA AGONISM AND THE ANTIHYPERGLYCEMIC ACTIVITY OF THIAZOLIDINEDIONES" JOURNAL OF MEDICINAL CHEMISTRY, vol. 39, no. 3, 2 February 1996, pages 665-668, XP000613613 cited in the application see page 665, right-hand column, "Discussion", lines 2-3, and page 666, Table I, compounds 1b and 2 --- -/-</p>	1-4,31

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

7 November 1997

Date of mailing of the international search report

13. 02.98

Name and mailing address of the ISA

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/13605

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	LENHARD J M ET AL: "ANALYSIS OF THIAZOLIDINEDIONE, BIGUANIDE AND RETINOID EFFECTS ON ADIPOGENESIS AND THE NUCLEAR RECEPTORS PPARGamma AND RXR" DIABETOLOGIA, SUPPLEMENT, vol. 39, 5 September 1996, page A234 XP000613679 see abstract ---	1-4,6,8,17
P,X	WO 97 10819 A (LIGAND PHARM INC) 27 March 1997 see page 13, line 1 - page 16, line 19; claims 2-10,14 ---	11,19
P,X	WO 97 25042 A (SMITHKLINE BEECHAM PLC; SMITH STEPHEN ALISTAIR (GB)) 17 July 1997 see page 2, line 6-13 see page 3, line 22-26 see page 12, line 8-23 see page 12, line 32 - page 14, line 21; claims 1-4 see claim 13 ---	1-4,13,14
P,X	see claim 13 ---	43
X	WO 95 07694 A (SANKYO CO; GOODMAN HERBERT H (US)) 23 March 1995 see the whole document ---	1-4,6,13-16
X	WO 95 07697 A (WARNER LAMBERT CO) 23 March 1995 see page 8, line 9 - page 27, line 12 see page 27, line 13-20 ---	1-4,6,13-16
X	LEHMANN J M ET AL: "AN ANTIDIABETIC THIAZOLIDINEDIONE IS A HIGH AFFINITY LIGAND FOR PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARGAMMA)" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 22, 2 June 1995, pages 12953-12956, XP000577082 cited in the application see the whole document ---	1-4,6,14-16,43
X	KARAM J H: "TYPE II DIABETES AND SYNDROME X PATHOGENESIS AND GLYCEMIC MANAGEMENT" ENDOCRINOLOGY AND METABOLISM CLINICS OF NORTH AMERICA, vol. 21, no. 2, 1 June 1992, pages 329-350, XP000605976 see the whole document ---	1-4,6-10,14-18,31
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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 97/13605

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BARNETT ET AL.: "Effect of Clofibrate on Glucose Tolerance in Maturity Onset Diabetes" BRITISH JOURNAL OF CLINICAL PHARMACOLOGY, vol. 4, 1977, pages 455-458, XP002045778 see the whole document ---	10,18
X	DATABASE WPI Section Ch, Week 9335 Derwent Publications Ltd., London, GB; Class B05, AN 93-278192 XP002045779 & JP 05 194 209 A (GRELAN PHARM CO LTD) , 3 August 1993 see abstract -----	10,18

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/ 13605

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1 - 13, 31  
because they relate to subject matter not required to be searched by this Authority, namely:  
see attached sheet
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see attached sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1 - 20, 31, 43

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 97/13605

### FURTHER INFORMATION CONTINUED FROM PCT/ISA/

1. Claims: 1-20, 31, 43 (partially)

Method for treating or preventing Type 2 diabetes and compositions adapted for use in the said methods and method of preparing a compound for treating Type 2 diabetes

2. Claims: 1-20, 31, 43 (partially)

Method for treating cardiovascular disease with diabetic or pre-diabetic conditions, method of preparing a compound for treating cardiovascular disease with diabetic or pre-diabetic conditions

3. Claims: 21, 28, 35, 37, 44

Method for treating or preventing hypertriglyceridemia, for lowering the level of plasma triglyceride or for increasing the level of HDL cholesterol, method of preparing a compound for treating hypertriglyceridemia

4. Claims: 22, 30, 33, 38, 45

Method for treating or preventing hyperglycemia, for lowering the level of plasma glucose or for increasing glucose uptake, method of preparing a compound for treating hyperglycemia

5. Claims: 23, 29, 32, 34, 39, 46

Method for treating or preventing hyperinsulinemia, for lowering the level of plasma insulin or for increasing insulin sensitivity or for preventing insulin resistance, method of preparing a compound for treating hyperinsulinemia

6. Claims: 24, 36, 42, 47

Method for treating or preventing hyperfibrinogenemia or for lowering the level of plasma fibrinogen, method of preparing a compound for treating hyperfibrinogenemia

7. Claims: 25, 41, 48

Method for treating hypertension or for lowering the blood pressure, method of preparing a compound for treating hypertension

8. Claims: 26, 40, 49

Method for treating obesity or for lowering the body weight,

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 97/13605

FURTHER INFORMATION CONTINUED FROM PCT/ISA/

method of preparing a compound for treating obesity

9. Claim : 27

Method for treating syndrome X

The problem underlying the application is seen as the provision of compositions for treating or preventing symptoms associated with type 2 diabetes, see specification pages 1 to 3. The conditions other than type 2 diabetes which are recited in the claims are not necessarily related to type 2 diabetes. This would warrant an a priori objection to lack of unity.

The solution claimed is the administration of a PPARGamma agonist and a PPARAlpha agonist, wherein both agonists can be the same compound, see claim 4.

The document J. Med. Chem. 1996, 39, 665-668 discloses the antihyperglycemic compounds clofibric acid 1b and 4-[4-(4-chlorphenoxy)phenyl]-4-oxobutyric acid 2 which show agonistic activity both on PPARAlpha and PPARGamma. Therefore, the principle of administering a PPARAlpha and PPARGamma agonist for treating type 2 diabetes and related diabetic conditions is neither new nor inventive and hence is not capable of providing a single general inventive concept unifying the above-captioned groups of inventions.

The search has been carried out for the first invention mentioned in the claims, i.e. treatment of type 2 diabetes comprising administering a PPARGamma agonist and a PPARAlpha agonist. A comprehensive search for the other inventions would have caused major additional searching efforts.

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 97/13605

### FURTHER INFORMATION CONTINUED FROM PCT/ISA/

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 1, 14, 31, 43

because they relate to subject matter not required to be searched by this Authority, namely:

Claims Nos.: 1, 14, 31, 43

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

A compound is not sufficiently characterized by its mechanism of action, as is the case for the expressions "PPARalpha agonist" and "PPARGamma agonist". The said expressions would encompass well-known substances which are conventionally being used in the treatment envisaged but whose mode of action has not as yet been described, thus being directed to a discovery. On the other hand, the said expressions would be directed also to substances to be prepared in the future and fitting the claimed mechanism. Therefore, the number of compounds comprised within these terms is infinite (Article 6 PCT), and the search had to be restricted to the classes of compounds specifically referred to in claims 5, 6 and 10.

#### Remark :

Although claims 1-13 and 31 are directed to a method of treatment of the human or animal body by therapy, the search has been carried out and based on the alleged effects of the composition.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. Int'l Application No

PCT/US 97/13605

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